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The Nature of Chromatin Changes in Colchicine-induced Variants in Sorghum

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THE NATURE OF CHROMATIN CHANGES IN COLCHICINE-INDUCED VARIANTS IN SORGHUM

By

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This thesis is approved as a creditable independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

The induction of true breeding variants with unchanged chromosome number in a variety of Sorghum vulgare Pers. was reported by Franzke and Ross (5). These variants resulted from colchicine treatment of seedlings in a variety which was known to breed true. The authors proposed that variant plants may have originated from a haploid chromosome complement formed by somatic reduction, which was later restored to the diploid condition. Such a cell by virtue of its genetic constitution or its position in the meristematic region may be able to initiate a new growing point having homozygous diploid tissue.

This proposal in itself does not explain the variation in plant type from the untreated full sib. These deviations may have arisen from chromosomal aberrations in the form of deletions, inversions or translocations within the chromosome complement. To determine if these chromosomal aberrations occurred a cytological study was made of the meiotic behavior in treated and untreated full sibs and F_1 hybrids involving variants induced by colchicine treatment.

LITERATURE REVIEW

Franzke and Ross (5) described the variation that arose when a true breeding grain sorghum variety was treated with colchicine. These variants gave no evidence of being polyploid contrary to the findings of Blakeslee and Avery (2) and many others. Variant plants were found that differed from the untreated grain sorghum type by having the open panicle, narrow leaf and the height characteristics

of the forage sorghum in its ancestry. The seed type in these plants resembled the forage sorghum but retained the free-threshing characteristic of the untreated full sib. This and other aberrant types were equally as fertile as their untreated full sibs.

Ross, Franks and Schuh (15) studied similar colchicine-induced variants out of a true breeding sorghum variety. These variants were found to breed true during four generations for the characters of height, stem diameter, number and length of leaves and panicle length. In line 10 segregation occurred for seed color. Comparisons were made by contrasting the variant to its untreated full sib where a difference in coleoptile color was noted in treated seedlings. The untreated material had uniformly red coleoptiles, line 10 expressed traces of red while line 12 and 15 were uniformly green. In this material highly significant differences for the characters studied were found between untreated and treated lines when each was taken as a unit.

These authors cited the agronomic importance of this induced variation since it would provide an opportunity for selection of desirable genotypes not previously recognized in the species. Because the variant is true breeding, desirable characteristics may be recognized and utilized immediately in a breeding program.

Franks and Ross (5) reported that no irregularities were discovered at meiosis. They proposed that reductional grouping of somatic chromosomes into haploid cells, containing concentrations of gene blocks from an ancestorial variety, could by splitting of these chromosomes form diploid tissue of new genetic constitution. This tissue by virtue of its genotype or position may be able to take over the

growing point and produce the variant plant type.

The evidence of somatic reduction of chromosomes in meristematic areas of plants was first reported by Huskins (7). The separation of the somatic chromosomes into two groups by the action of sodium malonate offered a possible new tool to the geneticist and plant breeder.

Wilson and Cheng (18) presented evidence from experiments using Trillium that reduction divisions resulting from treatments with sodium malonate, on root-tips undergoing mitosis, tend not only to separate the chromosomes into two groups approximately equal numerically; but also to separate homologues and thus bring about regular genetic segregation with much greater frequency than if segregation had been completely random.

Huskins and Cheng (8) found that the frequency of the reductional type of mitosis which occurs in root-tips can be greatly increased in cells of Allium cepa by growing the bulbs at low temperatures.

The fact that colchicine treatment resulted in similar reductional grouping was reported by Allen, Wilson, and Powell (1). Franzke and Ross (5) found reductional grouping of root-tip chromosomes in S. vulgare when treated for one hour in 0.5% colchicine. These findings lend credence to the supposition that a haploid chromosome complement formed by somatic reduction may form a restitution nucleus from which homozygous tissue will develop and take over the growing point.

Levan and Ostergren (9) noted that aberrant plant types with unchanged chromosome number have occurred after colchicine treatment. These were described as chromodilations. These authors also report

that when eggs of Strongylocentrotus were treated with a carcinogenic substance giant-formed larva resulted. It is stated that no mitotic disturbances were observed.

Somatic reduction would explain the true breeding nature of variants in sorghum but as pointed out by Ross, Franzke and Schuh (15) the appearance of new characters after treatment must result from a change in the chromatin. The study of pairing relationships of the pachytene chromosomes would afford the best opportunity for determining the nature of these changes.

The most detailed studies involving pachytene chromosomes have been made in Zea mays L. Mention is therefore made of the aberations found in this material. The chromosomes of this species have been found to be similar in many respects to those of S. vulgare. Rhoades (14) reported that the pachytene chromosomes of maize were individually recognizable by their relative lengths, the distinctive staining patterns, the position of the centromeres, and the deep-staining chromomeres adjacent to the centric region. The nucleolar bearing chromosomes remain associated with the nucleolus until its disappearance at the end of phophase. These chromosomes have been numbered according to their relative length, chromosome 1 is the longest and chromosome 10 is the shortest member of the haploid complement. The centromeres of non-homologous chromosomes were often observed stuck together at pachytene but becoming free at diplotene. It has been possible to discern the four chromatids which make up the homologous pairs at pachytene. The centromere of each chromosome does not divide until metaphase II. The contraction in length of the chromosome pairs is

first evidenced at zygotene and continues progressively through the meiotic prophase. Diplotene in this species is described as the two-by-two opening out of the paired homologues to form loops and nodes. The continued shortening of the chromosome takes place until at the close of diakinesis the nucleolus disappears, the spindles are formed and the chromosomes are oriented at the metaphase plate. The separation of the paired homologues into its two pair of chromatids begins with the anaphase movement.

McClintock (11) studied the nature of chromosomal alterations which were related to specific genetic changes induced by x-ray treatment. A deficiency which in this study was described as a terminal loss of chromatin was discovered when the paired zygotene chromosomes were observed. In plants where a deficiency had occurred half the pollen grains were defective. A short deficiency yielded pollen grains partially filled with starch whereas in a long deficiency the pollen grains were empty. When deletions (a loss of chromatin not terminal) occurred, the synapsis of the normal chromosome and its homologue resulted in a loop of unpaired chromatin in the normal homologue. This region represented the portion lost as a result of treatment. These fragments were lost when the acentric chromatin did not function at the anaphase movement.

A study which included ring-shaped chromosomes was made by McClintock (12). It was found that the ring-shaped chromosome resulted when a deletion occurred including a portion of the spindle fiber attachment region. Both sections of the broken spindle fiber

attachment region were capable of functioning in the spindle figure and, consequently both the rod-shaped and the ring-shaped chromosomes perpetuated themselves. A chromosomal aberration of this type differed from the break described before since continued functioning of the ring fragment allowed a normal phenotypic expression in the plant.

McClintock (11) reported that in the case of inversion of large portions of a chromosome configurations in the nature of cyclic figures resulted when pairing occurred between the normal chromosome and its rearranged homologue.

Reciprocal translocations have been found to occur naturally and as a result of x-ray treatments. Pairing involving reciprocal translocations results in associations between two or more pair of pachytene chromosomes. In these studies all translocations were found to be reciprocal.

The loss or gain of chromatin has given rise to phenotypic responses in polyploid cereals. Huskins (6) demonstrated that fatuoid, speltoids and related oat and wheat mutants arise through chromosome aberrations.

The chromosome number of S. vulgare has been reported as 2 n = 20, Darlington and Janaki Ammal (4). Longley (10) described the pachytene chromosomes as having a very distinct clear region of spindle-fiber attachment with a deeply staining area on either side. The staining quality decreased toward the ends of the chromosomes. The ten chromosome pairs were found unmarked by knobs. The longest chromosome was observed to be attached to the nucleolus near its midpoint, a short

distance from the spindle-fiber attachment. He pointed out that length alone is not always an entirely satisfactory method of identifying chromosomes because of the difficulty of having all chromosomes and all parts of chromosomes equally extended.

Kidd, H. J. (from conversation, June 1953) has observed that the chromosomes of S. vulgare are frequently found connected by strands of material of unknown origin. These have been termed "secondary-associations" and become visible during diakinesis and metaphase I.

MATERIAL AND METHODS

Two varieties of S. vulgare, Experimental 3 and Experimental 1 were used in this study. In addition six other lines were used as males in crosses with a colchicine induced variant from Experimental 1.

Experimental 3 resulted from crosses made in 1932 between the variety Day, a late maturing dwarf grain sorghum, with Black Amber Cane and Sudan grass. Out of each of these crosses a segregate resembling Day was selected. In 1939 a cross was made between these lines. From their progeny, through continued selfing and selection the true breeding Experimental 3 was produced.

In the winter of 1951-52, 15 seedlings were grown from selfed seed of one plant of the Experimental 3 variety. Eight seedlings were selected at random as untreated controls from which were derived lines 1 through 8, while seven seedlings were treated and gave progeny forming lines 9 through 15. All plants were grown to maturity in the green house and selfed seed harvested. In the spring of 1952 the original 15 plants and their selfed progeny were moved to the field

for study.

In the evaluation of the response of plants 9 through 15 to colchicine treatment it was found that all plants developed the rosette stage described by Franake and Ross (5). Neither the treated plants numbered 9, 11, 13, and 14, nor their progeny showed gross morphological differences in the field when compared to the untreated full sib. Line number 10 segregated for several characters indicating it was a possible chimera. Lines 10, 12, and 15 differed widely from untreated full sibs. Line 15 had the narrow leaves, open panicle, and the height characteristic of its Sudan grass ancestor.

Experimental 1 is a true breeding variety selected from a cross made in 1939 between a line derived from Dwarf Feterita X Dwarf Freed and the variety Grohama. In 1947 seedlings of this material were treated with 0.5% colchicine in lanolin. Progeny of this material was again treated in 1948. The "rat-tail" variant resulting from this treatment was an extreme dwarf which was designated OB31. The head type of this line was cylindrical, compact and slender hence the name "rat-tail".

In 1949 lines from the breeding nursery designated 20, 30, 40, 50, 60, 70, and 80 were used as males and crossed with OB31. Each of these lines had been derived from plants treated with colchicine but they did not express gross variations from their untreated full sibs. Line 60 and OB31 were derived from treated full sibs. In each case the line was tested and found to breed true.

Material for the study of meiosis in Experimental 3 was collected in the field in the summer of 1952. To obtain similar material from

Experimental 1 plantings were made in the greenhouse. The material making up Experimental 3 was returned to the greenhouse in the fall of 1952 where crosses between treated and untreated lines and within treated lines were made. The F_2 seed was planted in a soil bed in the greenhouse and material for the study of meiosis was collected early in the summer of 1953.

Material collected for smears was fixed in Farmer's fluid as described by Smith (16) and stored in a refrigerator.

Smears were made by a modification of the technique described by Smith (16). In all cases a propio-carmine stain was used. This was prepared by adding 0.5 grams of carmine to 100 c.c. of 45% propionic acid, boiling the mixture with a reflux condenser for about two hours, and filtering when cool. No iron was used with this stain. Slides were made permanent in two ways. The McClintock (13) method of preservation was used for about one half of the slides while a venetian turpentine seal technique as described by Wilson (17) was used on the other slides. The chromosomes were equally well preserved and discernible regardless of the method by which the slides were made permanent.

This material was examined with 16 mm and 2 mm microscope objectives and 5X, 10X and 20X oculars.

In addition to visual observation the chromosomes were studied and recorded by means of camera lucida drawings at approximately X 3300 magnification. The length of the chromosomes was determined by means of a map measuring device which recorded linear distance in inches to 0.05 parts of an inch. All measurements were recorded to

the nearest tenth of an inch and converted to microns.

The photomicrographs were made with 2 mm objective, 12.5 X ocular and 20 inch bellows extension on the camera. The images were recorded at a magnification of approximately X 2700 on 5" X 7" Contrast Process Ortho and Contrast Process Panchromatic Kodak films, developed in D-11 developer. A Wratten B filter No. 58 was used at the light source. The color photograph was made on Ansco Color film.

EXPERIMENTAL RESULTS

Experimental 3

To establish the meiotic behavior of the material being studied a careful examination of the chromosomes from all lines involving Experimental 3 was undertaken. From the permanently preserved slides, camera lucida drawings and photomicrographs were made of cells in which the entire complement of the 10 pachytene chromosomes were discernible.

These chromosomes were scrutinized for the presence of unpaired areas, loops, deficient terminal sections or multiple associations among pairs. In all material derived from Experimental 3 instances of unpaired short terminal sections were observed but since these were not found in every cell and were not always associated with the same chromosome they could not be considered as indicative of lack of homology.

The presence of incomplete pairing of internal sections of the chromosomes was found less frequently than the forked-end type of unpaired area. This failure to pair did not resemble the loop

described by McClintock (11) since the unpaired areas of each homologue appeared to be of equal length. The lack of paired sections was not associated with any particular chromosome.

Untreated full sib

No abnormalities were found in the meiotic chromosomes from the 8 untreated full sibs. The total complements of pachytene chromosomes from 11 cells were recorded and studied. In addition a much larger number were carefully studied but since certain areas were indistinct complete records were not possible.

Figure 1 through 3 illustrates the type of pachytene material studied in the untreated Experimental 3. In Figure 1 a short unpaired area is evident.

In the study of later stages of meiosis the complete absence of multiple associations among chromosome pairs and subsequent bridging in either the anaphase I or anaphase II movement was established.

In Figure 8 a typical metaphase I plate of S. vulgare is seen. This is a photomicrograph of Experimental 3, line 1.

Treated full sibs

Chromosomes of lines 9 through 15 were studied at all stages. When pachytene chromosomes were drawn and evaluated no instance was found in which the chromosomes differed from chromosomes in the untreated material. The frequency of unpaired ends or unpaired internal sections had not been increased, and later stages of meiosis were in each case regular. Special attention was paid to anaphase movements to determine if any fragments were being left behind but no indications of such irregularities were noted.

Figure 4 shows complete pairing of the chromosomes.

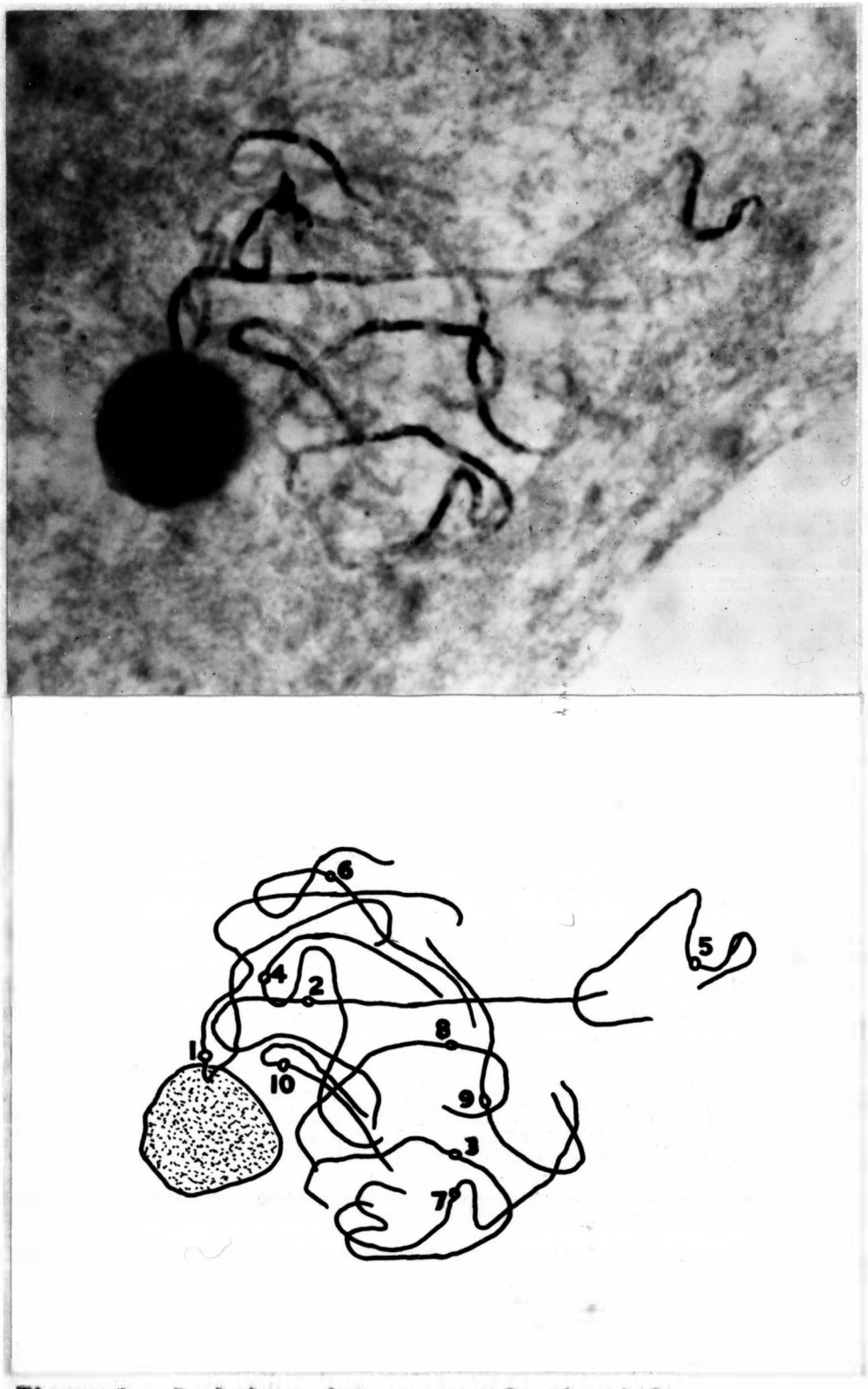


Figure 1. Pachytene chromosomes of untreated line 4 from Experimental 3. An unpaired area is seen in chromosome 5.

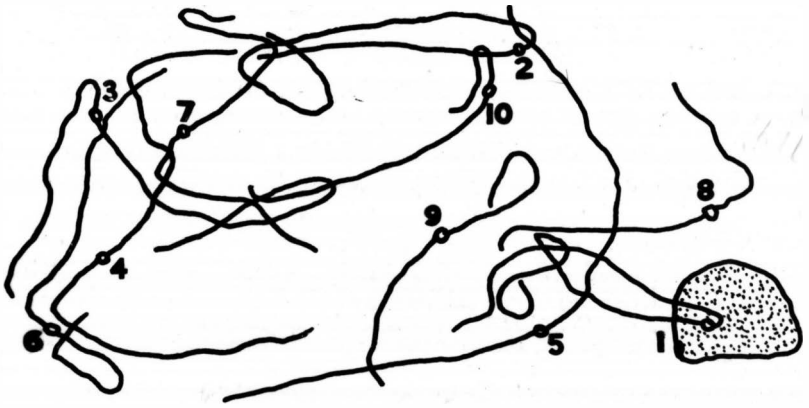
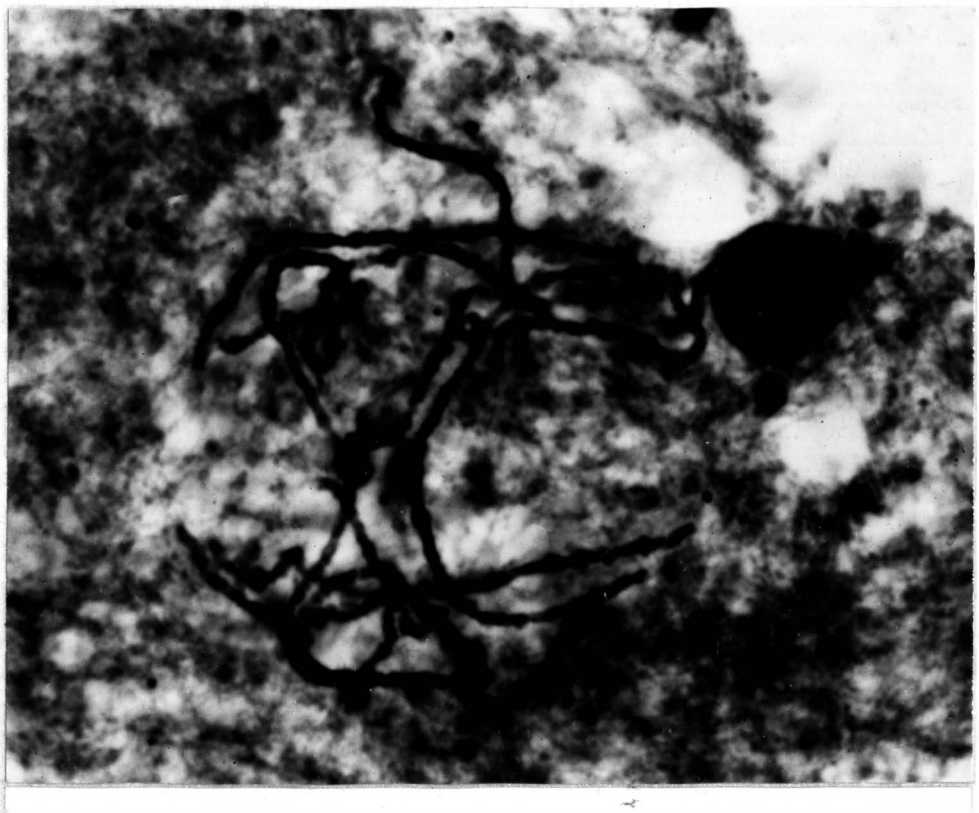


Figure 2. Pachytene chromosomes of untreated line 5 from Experimental 3.



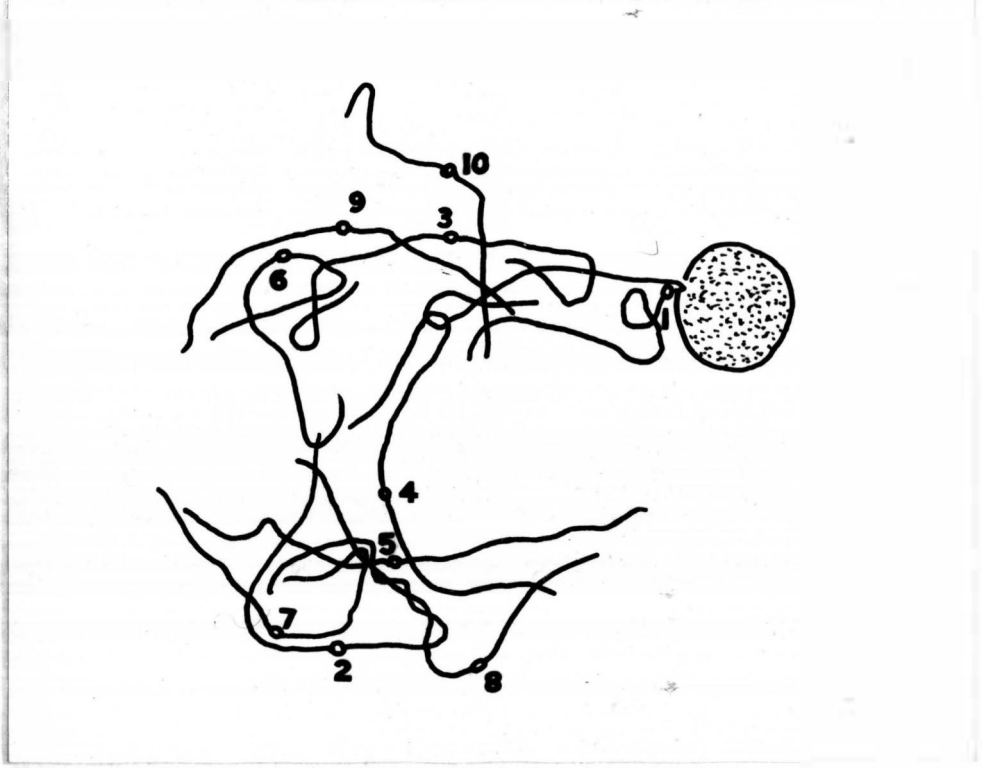
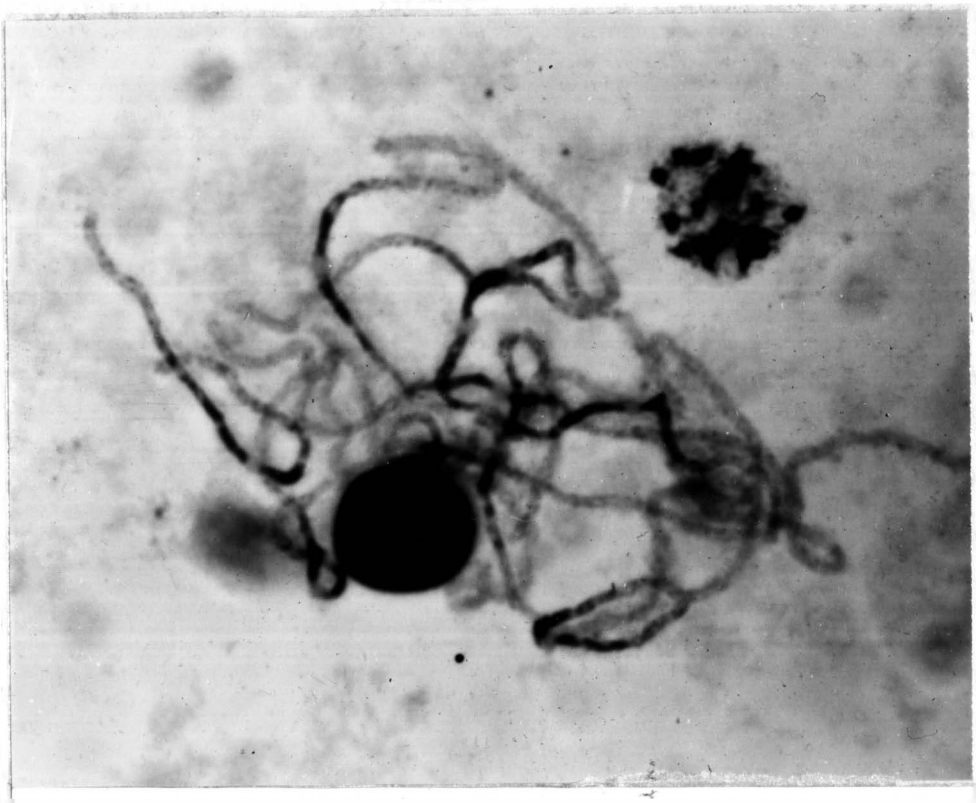


Figure 3. Pachytene chromosomes from untreated line 6 from Experimental 3.



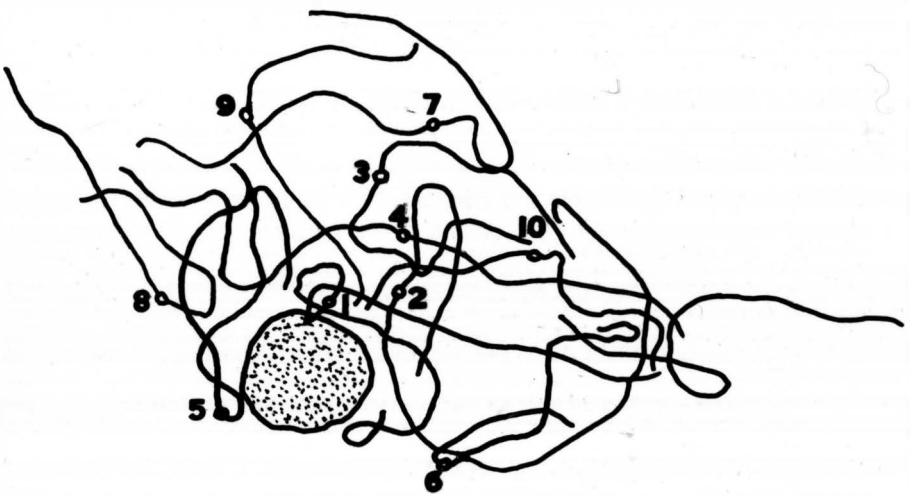
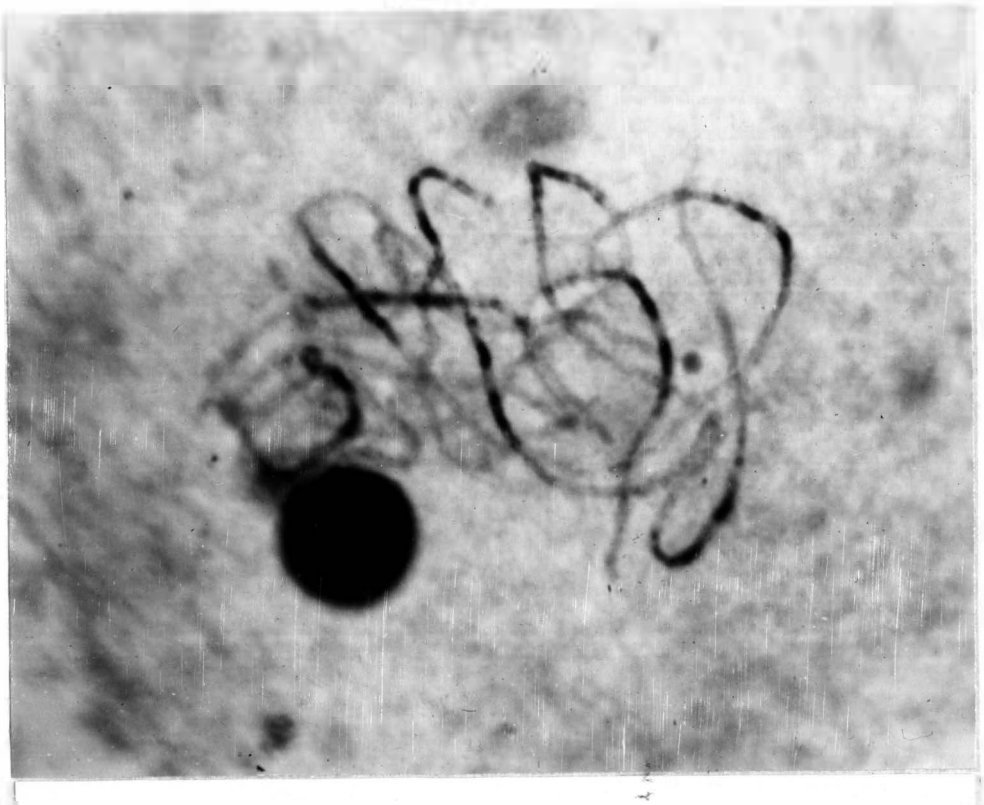


Figure 4. Pachytene chromosomes from treated line 15 from Experimental 3. A piece of chromatin from an adjacent cell is seen between chromosomes 6 and 10.



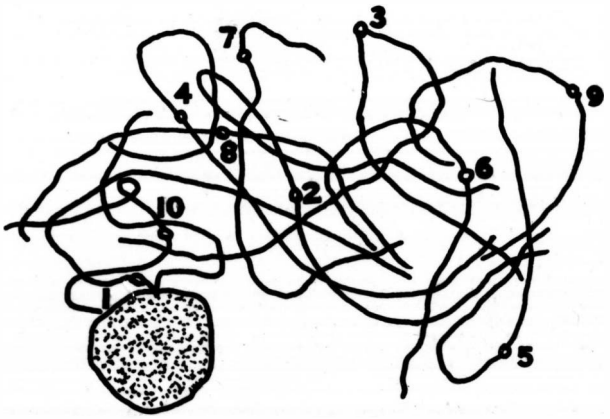


Figure 5. Pachytene chromosomes from an F_1 hybrid of Experimental 3 between untreated line 4 and treated line 15.

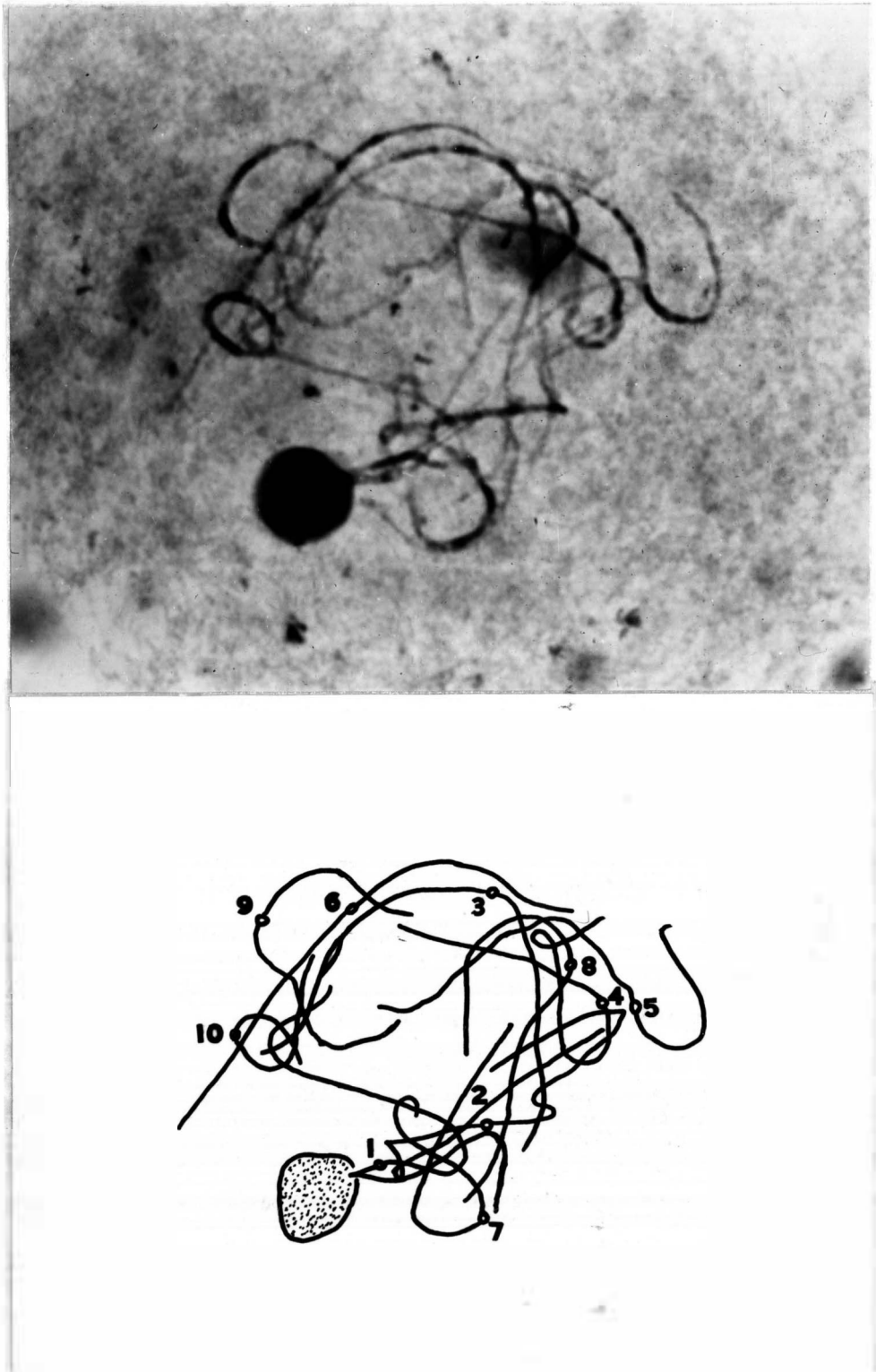
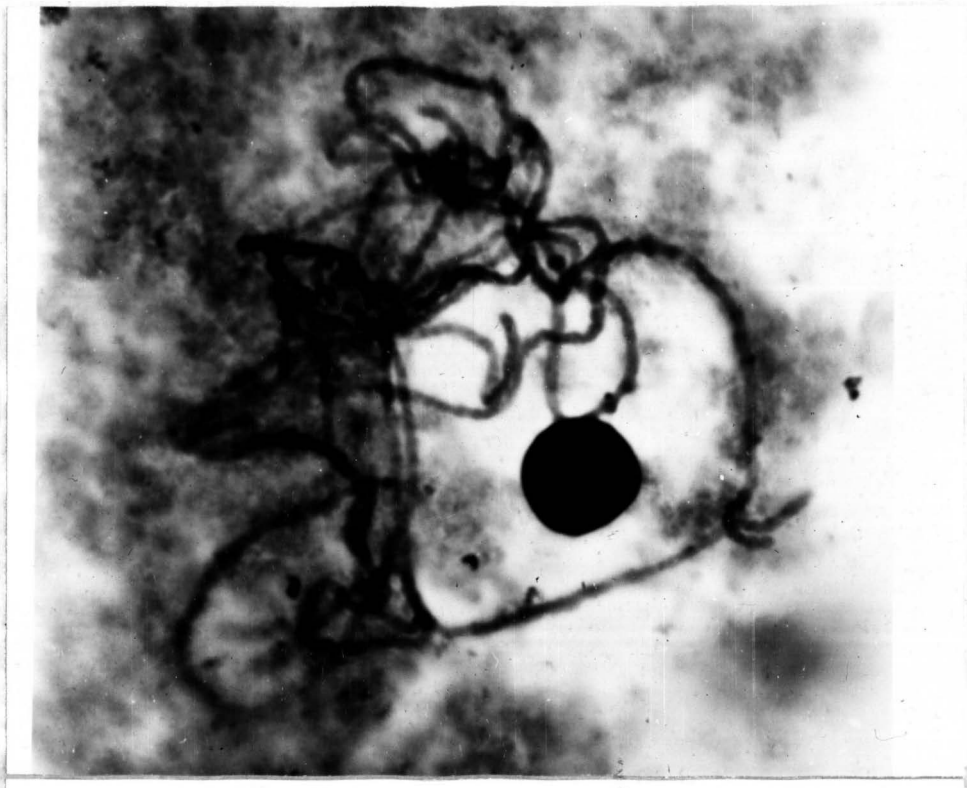


Figure 6. Pachytene chromosomes from an F_1 hybrid of Experimental 3 between untreated line 6 and treated line 15. A relatively large unpaired area is seen in chromosome 3.



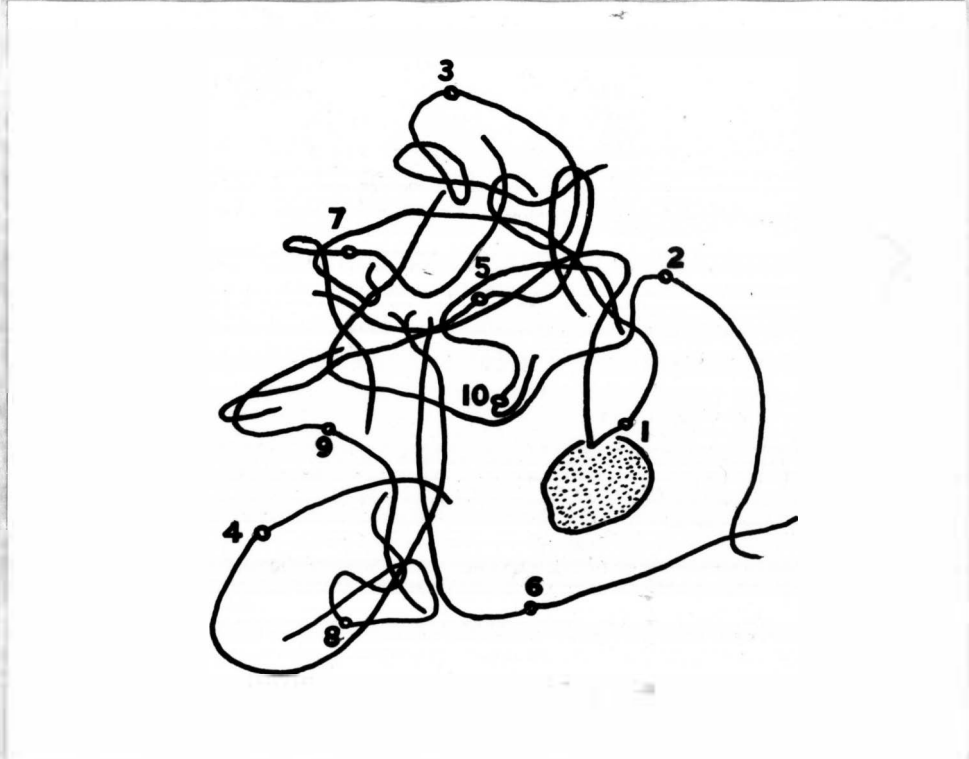


Figure 7. Pachytene chromosomes from an F_1 hybrid between treated variant lines 12 and 15 from Experimental 3.

Figure 8. Metaphase plate of untreated line 1 from Experimental 3. "Secondary-associations" are visible.

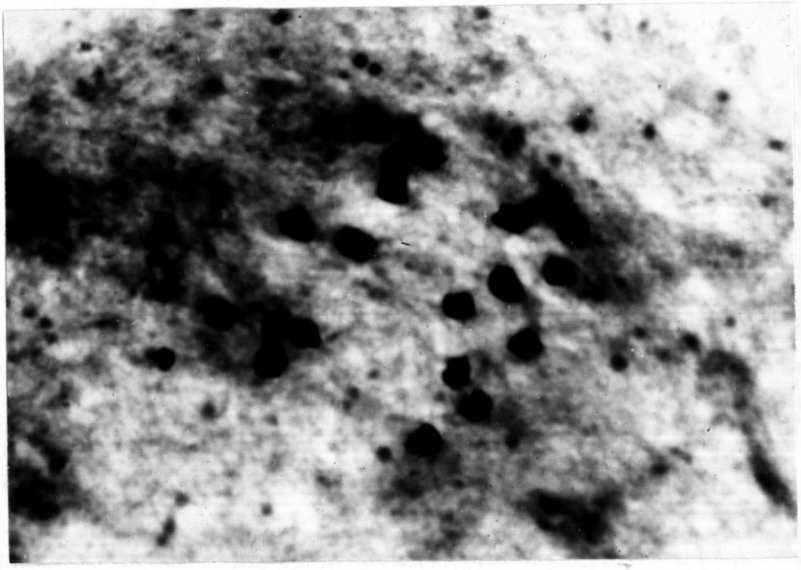


Figure 9. Anaphase movement in line 6 from Experimental 3.

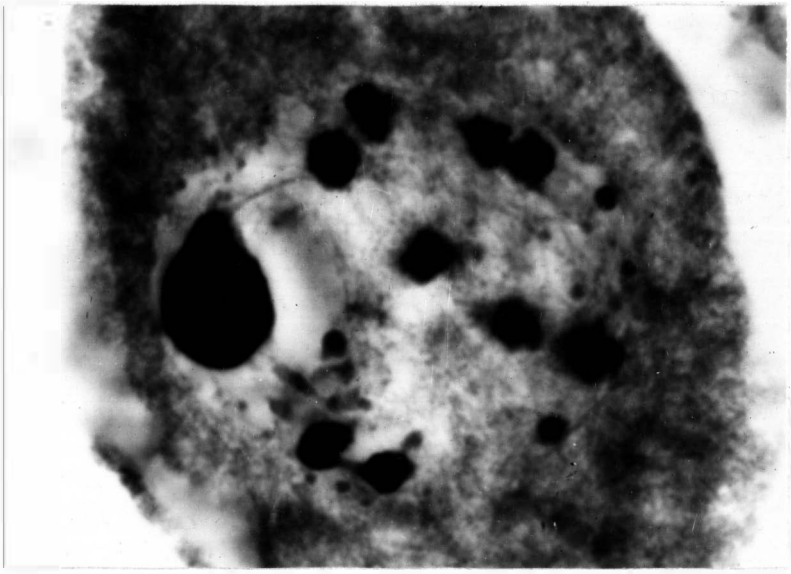


Figure 10. Diakinesis in line 15, a treated variant from Experimental 3.

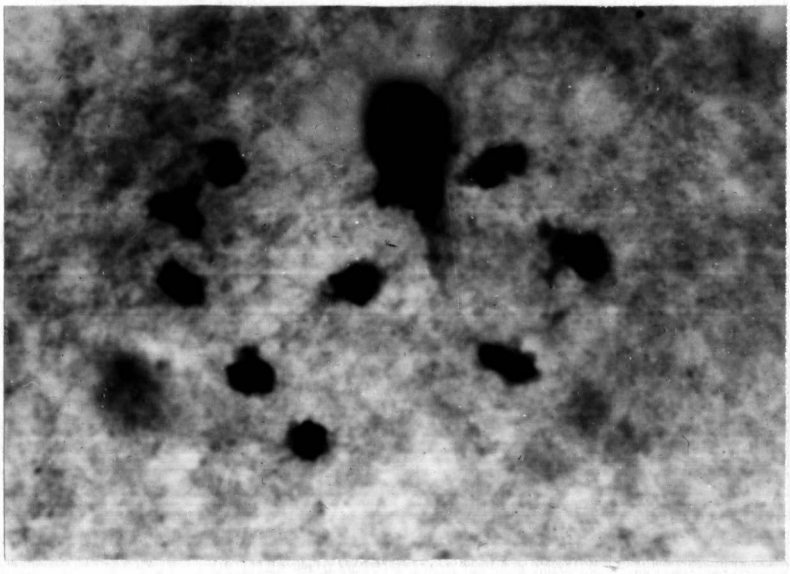


Figure 11. Diakinesis in an F_1 hybrid between untreated line 6 and treated line 15 from Experimental 3.

F1 hybrids of treated and untreated full sibs

Meiosis in the F_1 hybrids of the crosses of lines 4 X 10, 4 X 15, 5 X 15, 6 X 15, and 12 X 15 was studied. Figures 5 through 7 illustrate the normal pairing found at pachytene in these crosses. In Figure 6 a relatively large unpaired area is visible but this cannot be considered as evidence of chromatin rearrangement since areas of this type were found in the untreated lines.

In studies of the diakinesis, metaphase and anaphase stages normal arrangements and movement of the chromosomes were found. In Figures 8 through 11 the regularity of these stages of meiosis in the untreated, treated and crossed lines is illustrated.

Experimental 1

This experimental breeding line was of special interest since the "rat-tail" variant deviated widely from the untreated full sib and also since progeny from crosses involving this variant gave large-seeded segregates (unpublished data).

Meiosis in the variant OB31 was studied. Similar studies were made in lines 20 through 80, which were crossed with OB31 and in F_1 hybrids resulting from these crosses.

In this material short unpaired areas were found in pachytene chromosomes from all lines. These unpaired areas appeared to be the same as those found in Experimental 3.

Line OB31

In Figure 12 normal pairing of OB31 chromosomes is seen at pachytene. Since material available of this line was limited, no cells were obtained in which camera lucida drawing could be made

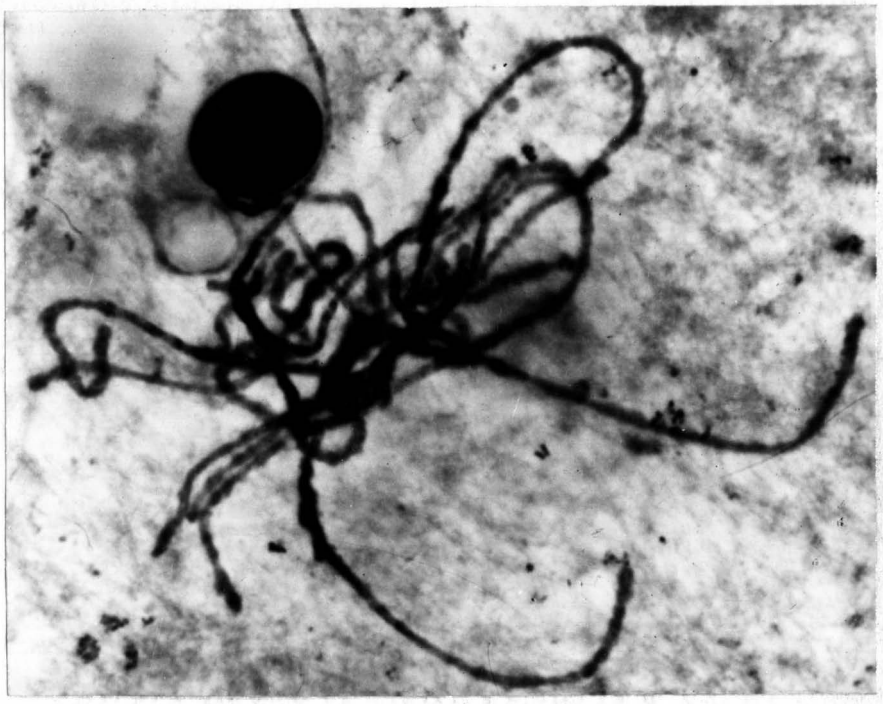


Figure 12. Pachytene chromosomes from OB31, a colchicine induced variant from Experimental 1.

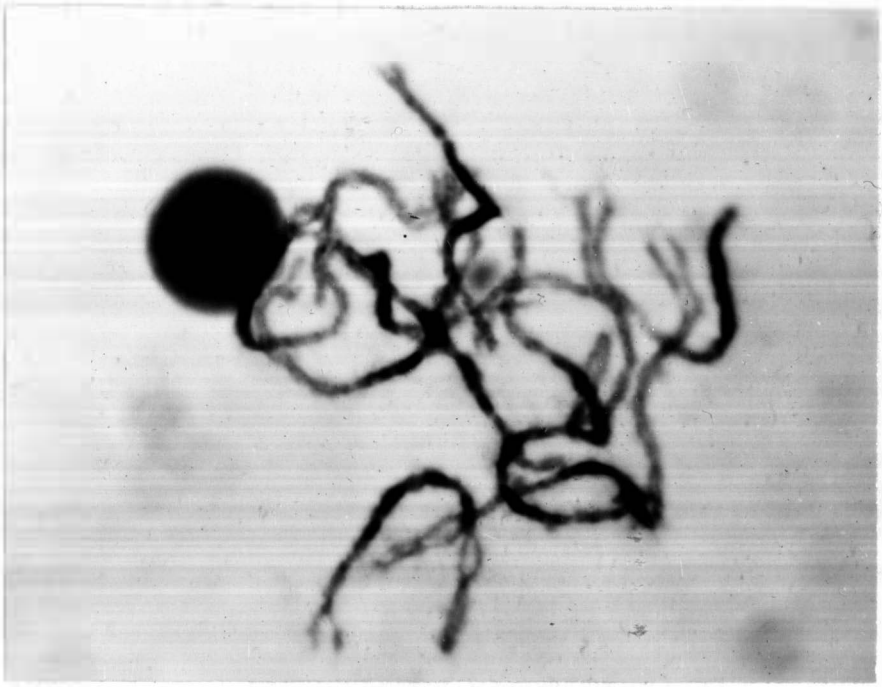
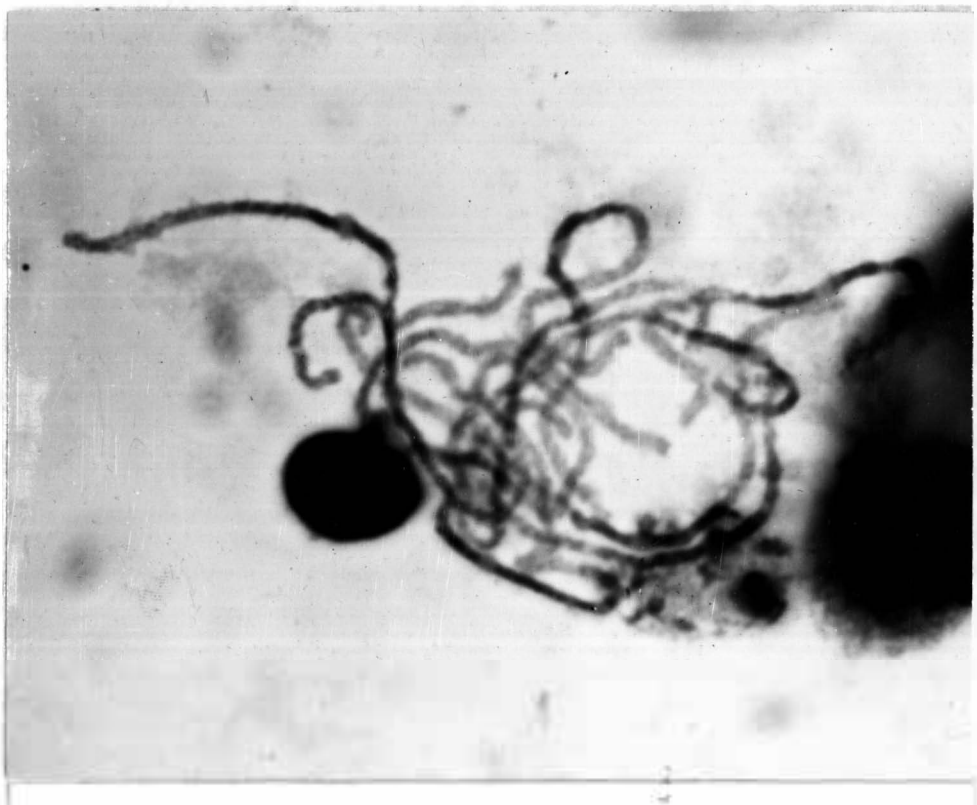


Figure 13. Pachytene chromosomes from line 50 showing large unpaired areas.



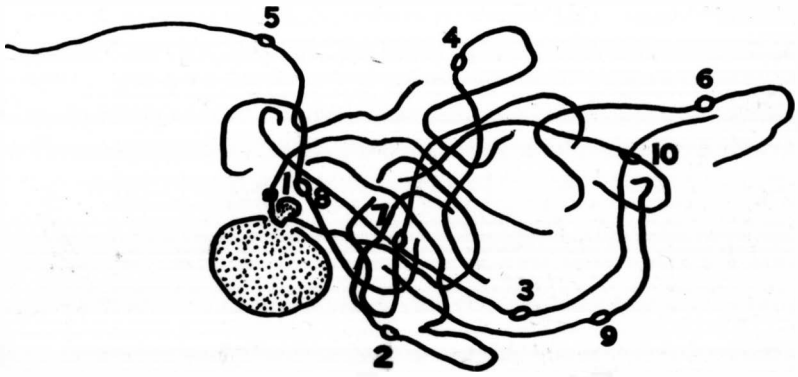
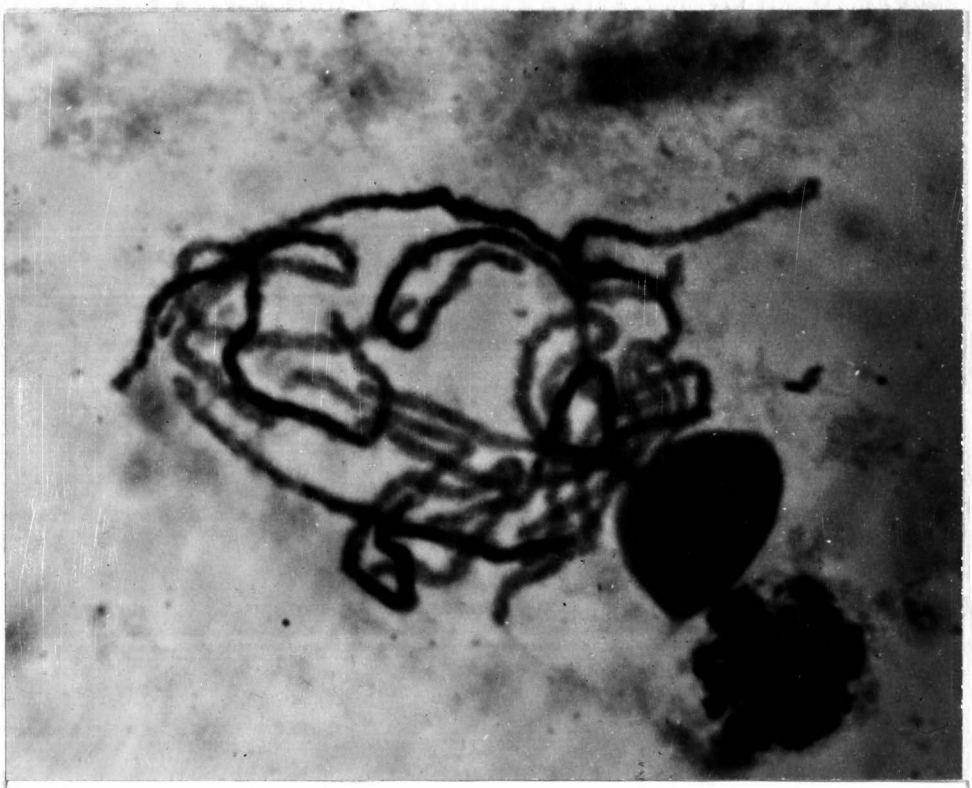


Figure 14. Pachytene chromosomes from line 60. This line and line OB31 were obtained by treatment of full sibs.



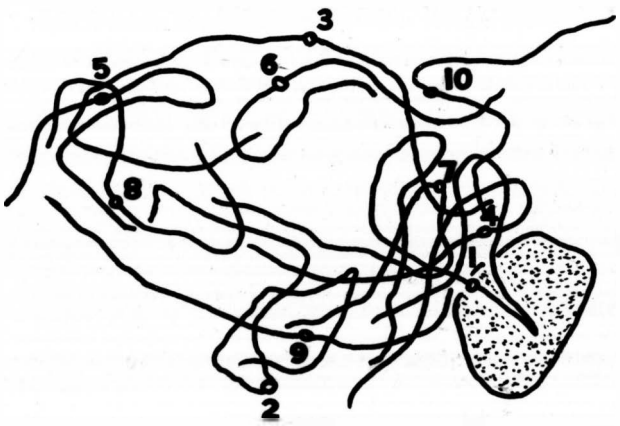
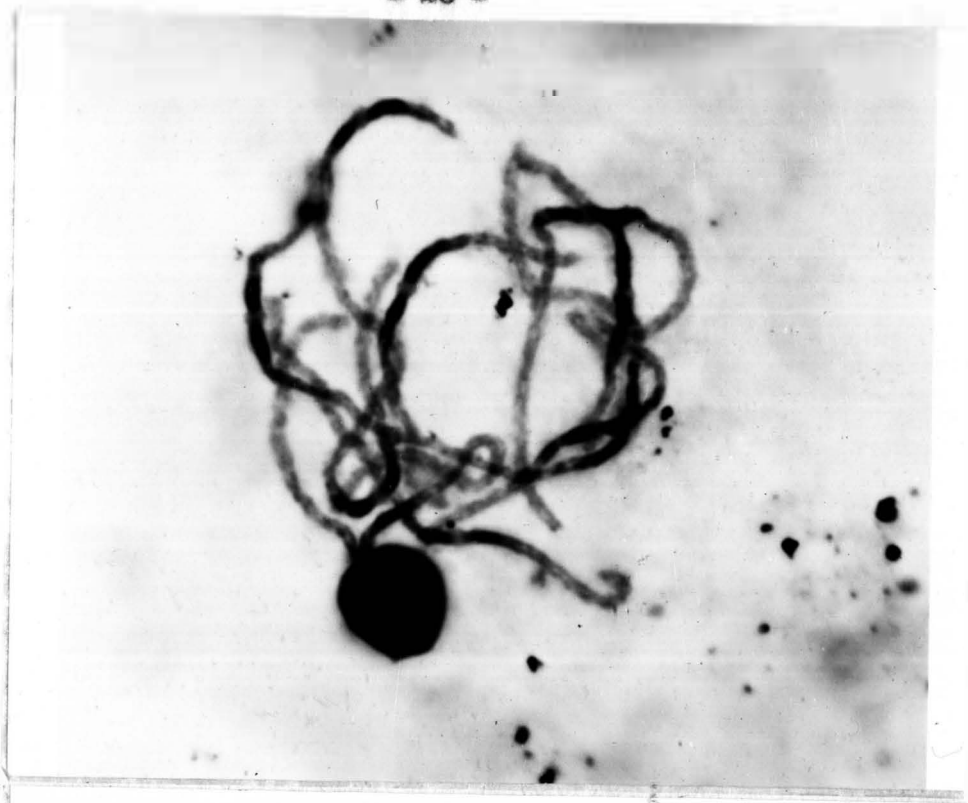


Figure 15. Pachytene chromosomes from another cell of line 60 described in Figure 15.



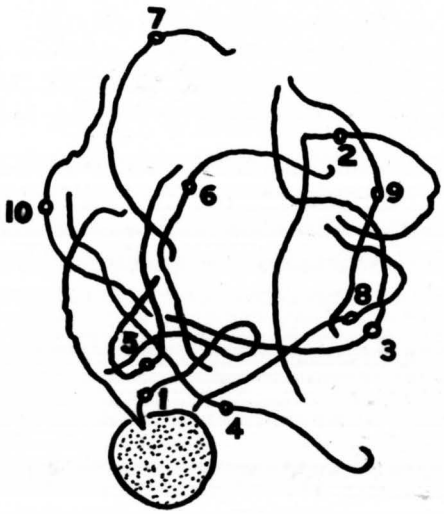
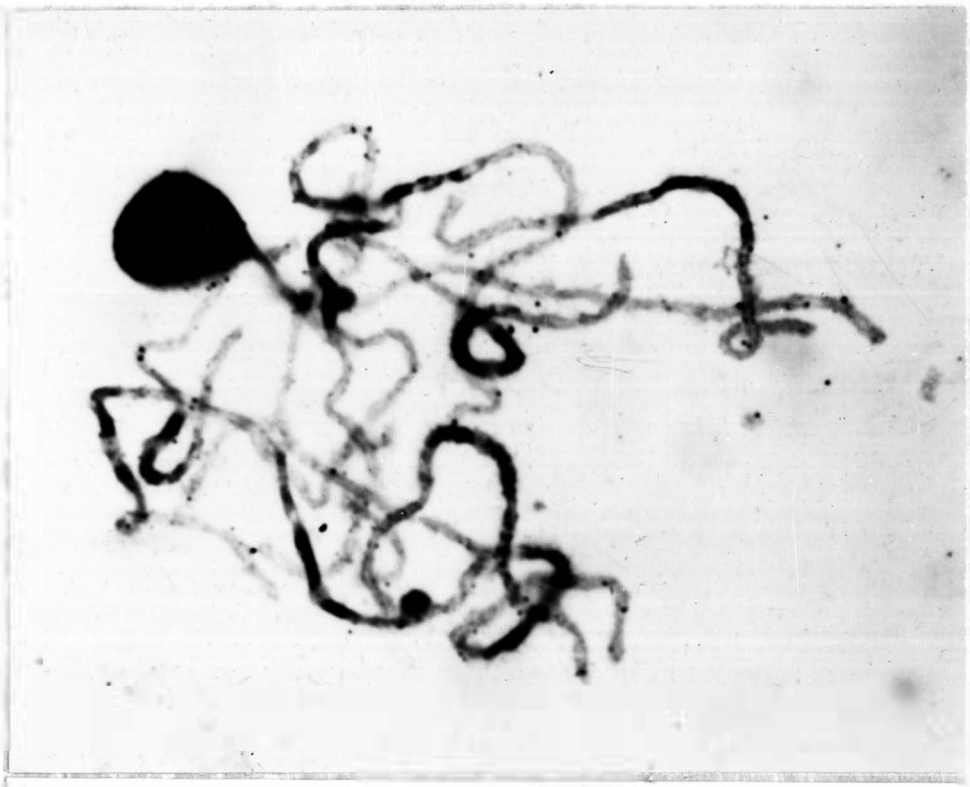


Figure 16. Pachytene chromosomes from line 80. This material was used as a male when crossed with OB31.



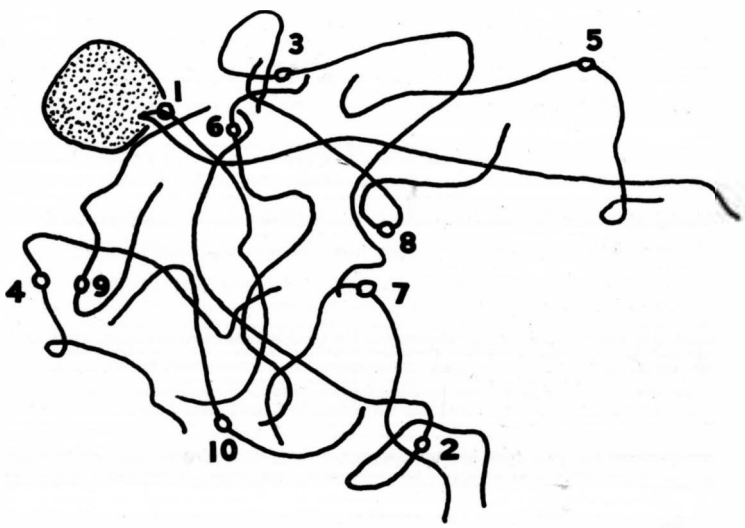
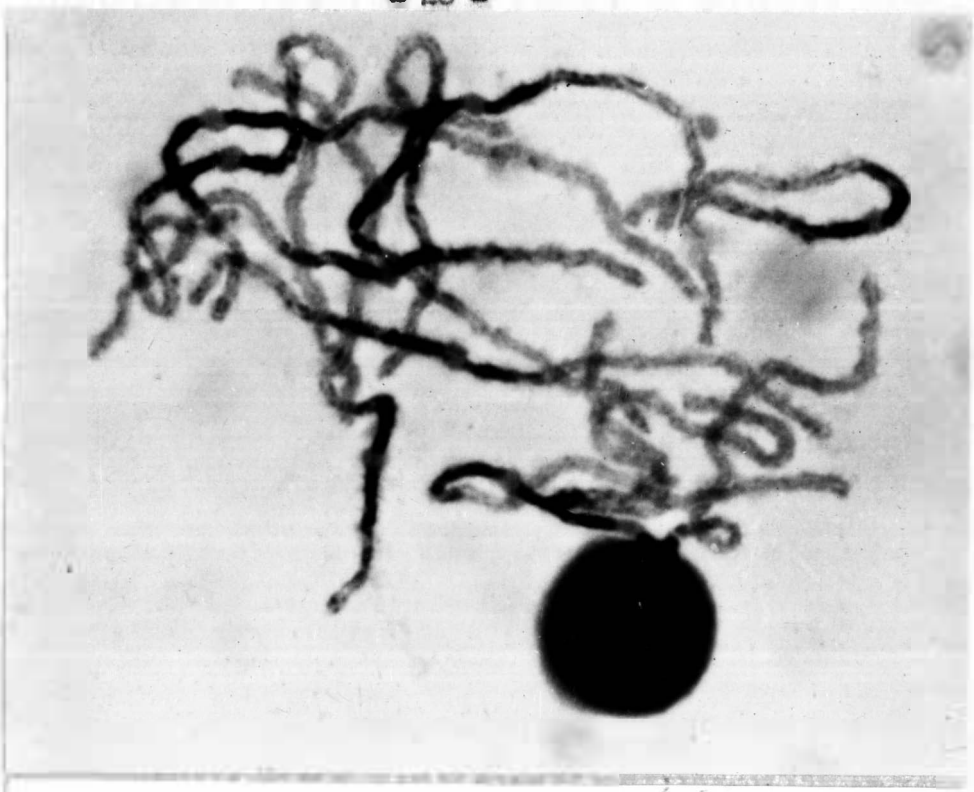


Figure 17. Pachytene chromosomes from an F_1 hybrid between OB31 and line 30.



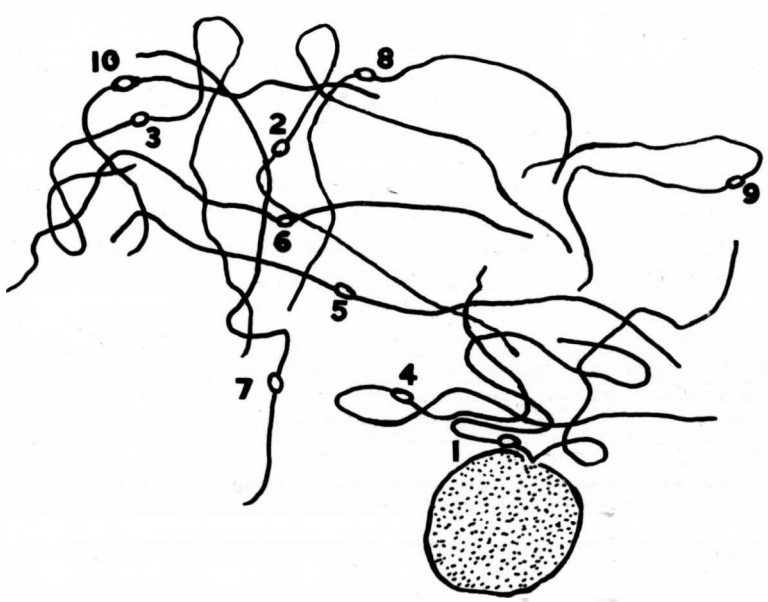


Figure 18. Pachytene chromosomes from an F_1 hybrid between OB31 and line 30.

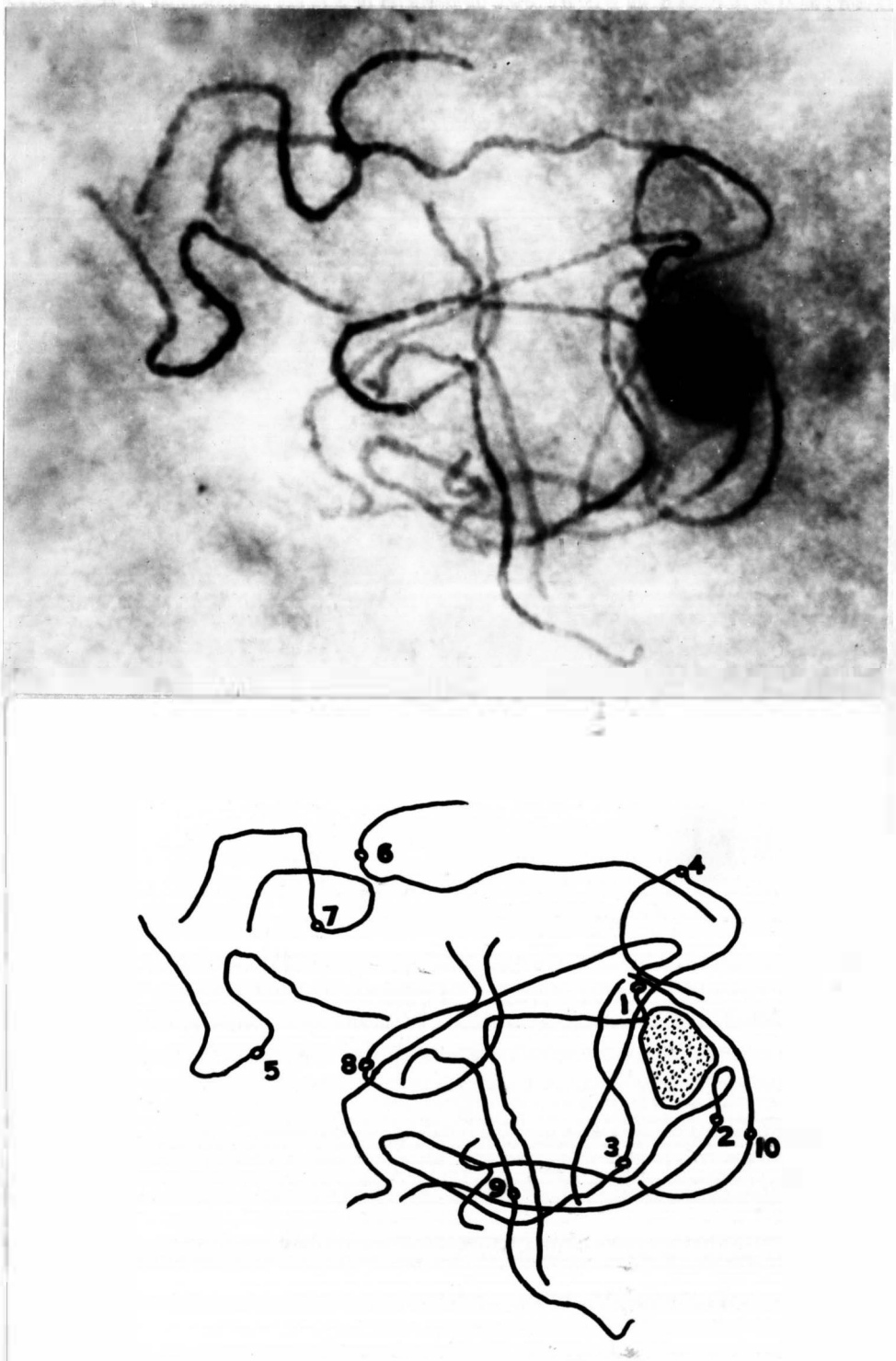
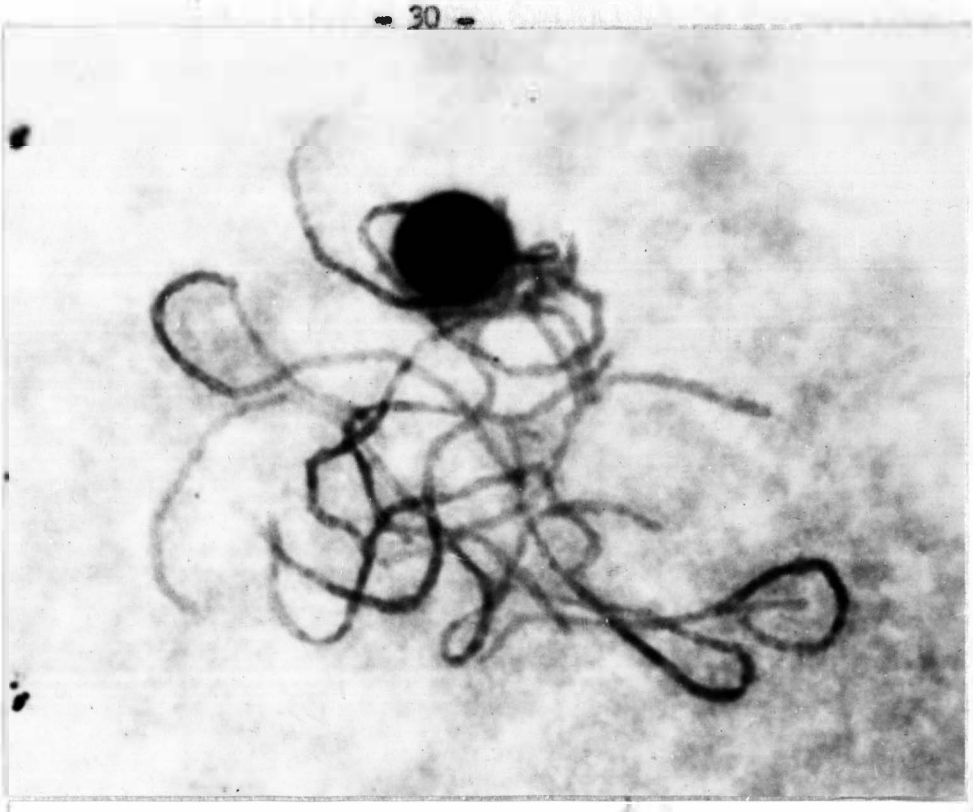


Figure 19. Pachytene chromosomes from an F_1 hybrid between OB31 and line 80.



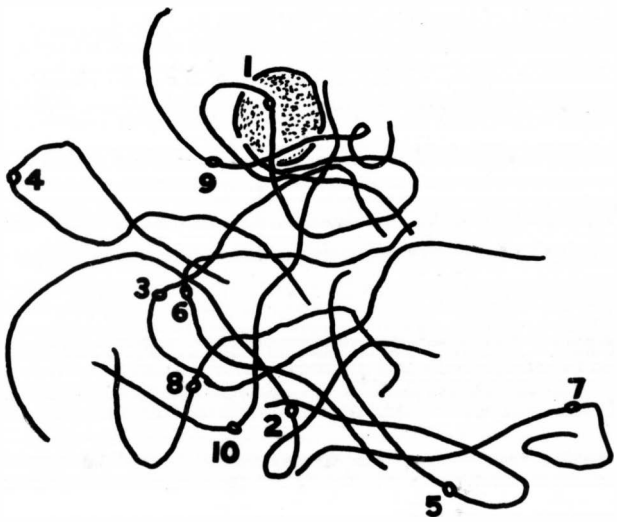


Figure 20. Pachytene chromosomes from an F_1 hybrid between OB31 and line 80.

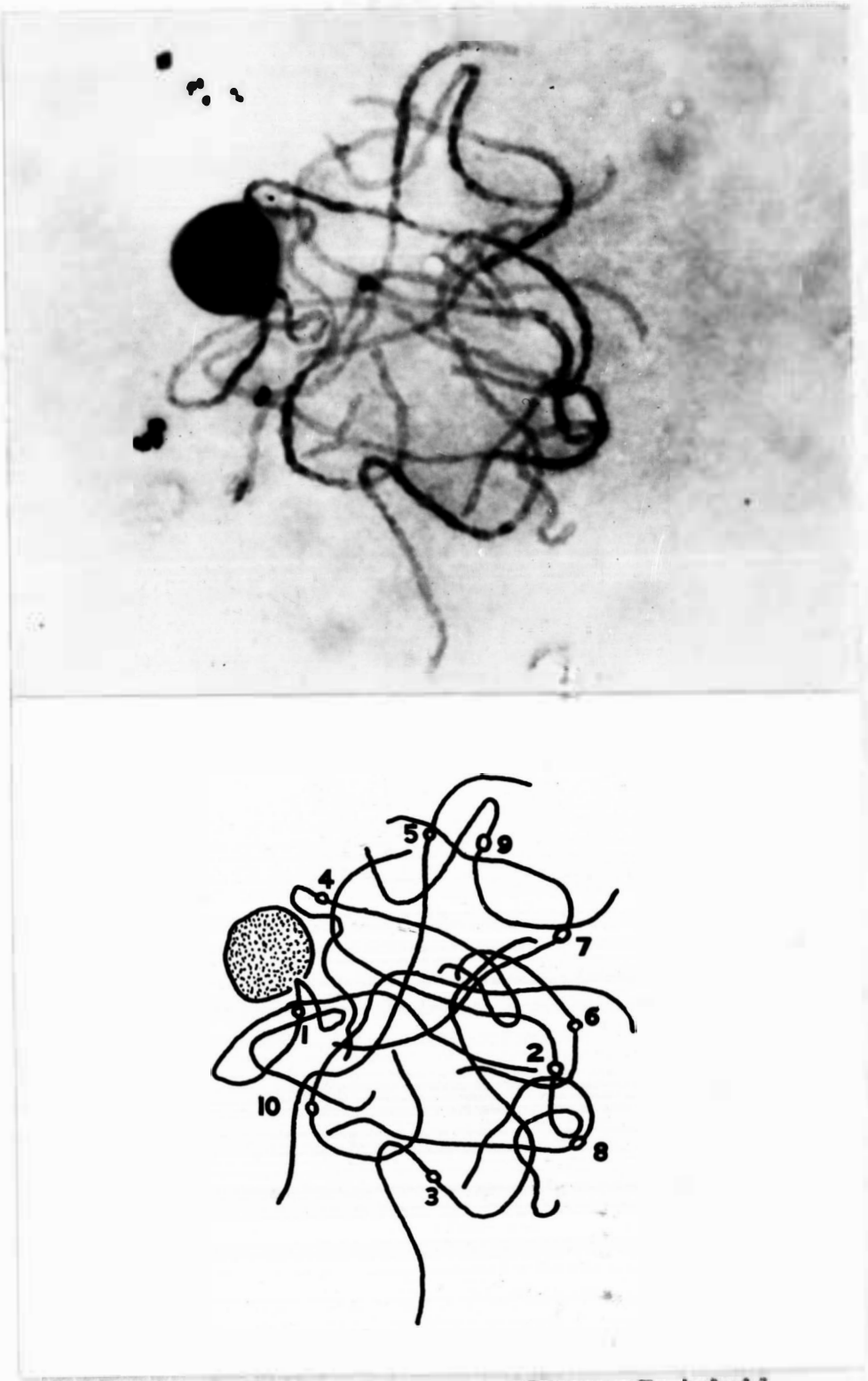


Figure 21. Pachytene chromosomes from an F_1 hybrid between OB31 and line 80.

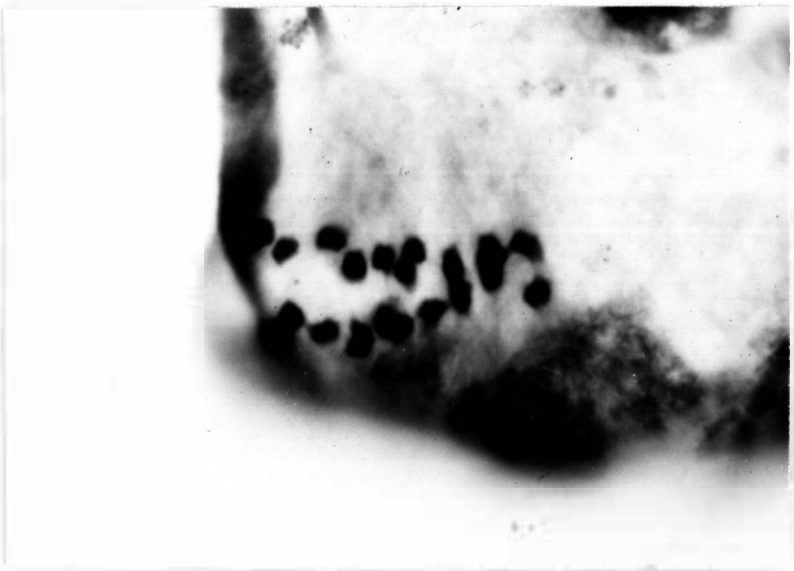


Figure 22. Anaphase movement in a cell from line 50.

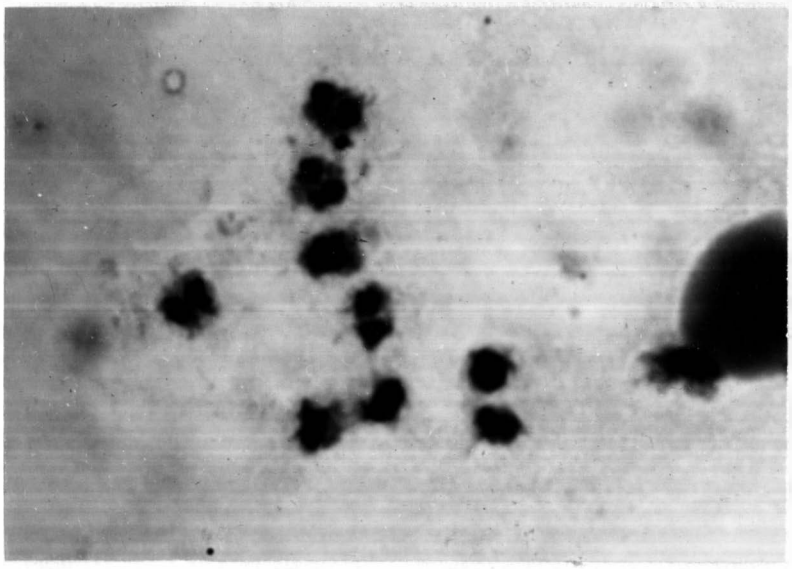


Figure 23. Chromosomes at diakinesis from line 60.

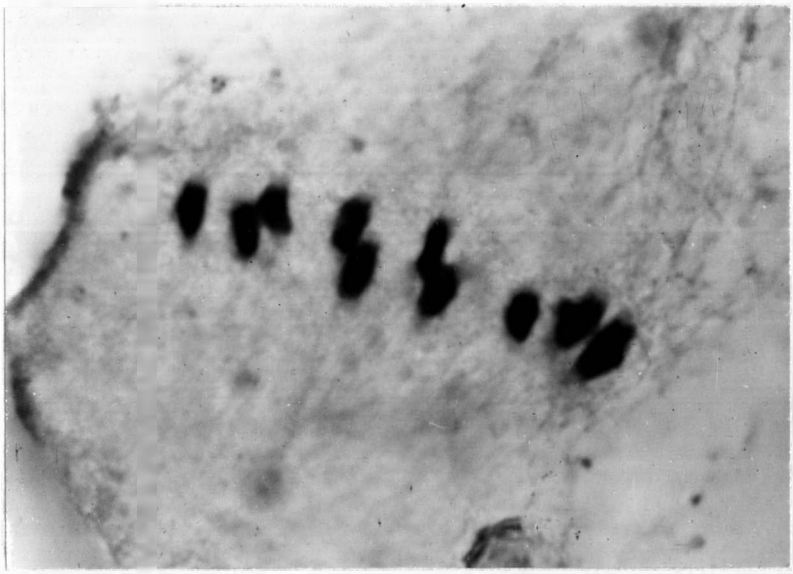


Figure 24. Metaphase plate from OB31.

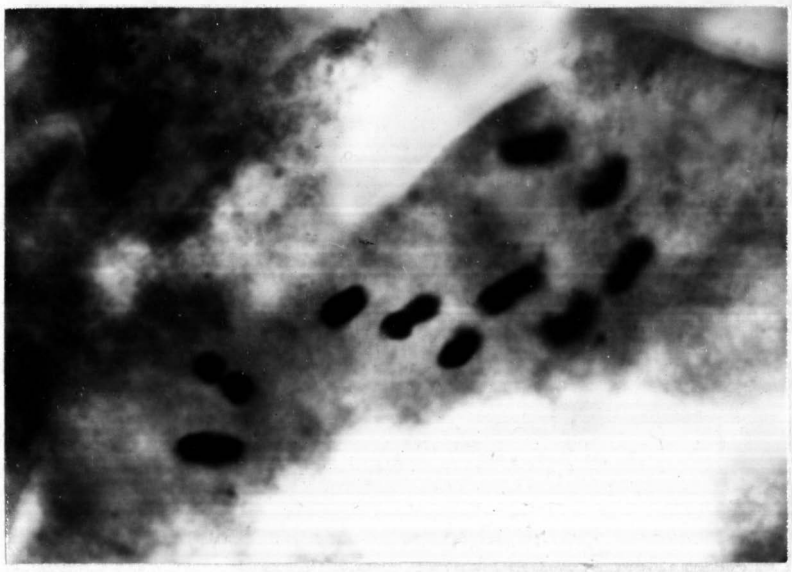


Figure 25. A polar view of the metaphase plate from an F_1 hybrid between OB31 and line 60.

For each chromosome pair the mean length of the arm and the ratio of the long arm to the short arm was calculated. These comparisons are presented in Table 1. Since no crosses were available between these varieties, which would indicate the homology of the chromosomes, an estimate was obtained by comparison of ideograms constructed from the data in Table 1. A distinct similarity was found between the ideograms constructed from these units. Large amounts of variation were found when individual cells were studied by this method. The differential contraction of chromosomes and parts of chromosomes probably accounted for the variation. This variation was illustrated when in 6 out of the 29 cells the nucleolus was found on the long arm of chromosome number one rather than the short arm. A chromosome of medium length (probably number 6) was often observed to have a very short arm which was heterchromatic along its entire length. Since this shortening was not always found even within a single plant it is probable that a rapid contraction occurred within this arm during late pachytene. These discrepancies existed in the material from both varieties. Since the results indicated the lines to be similar, a composite ideogram (Figure 27) was constructed representing the relative mean length of each chromosome in the 29 cells compared.

Attractions described as "secondary associations" (Figures 8 and 23) were frequently seen at diakinesis and metaphase stages in treated and untreated material. Figure 26 illustrates an attraction between centromeres similar to those seen in corn. These associations did not persist at diakinesis.

When crosses between treated lines 12 and 15 of Experimental 3 were made it was noted that the red coleoptile characteristic of the

Table 1. A comparison of measured chromosome lengths and ratio of long to short arm from lines within Experimental 1, Experimental 3 and a composite of these.

Chromosome Number		Experimental 1		Experimental 3		Composite	
		Length of arm in microns (average of 12 cells)	Ratio of long to short arm	Length of arm in microns (average of 17 cells)	Ratio of long to short arm	Length of arm in microns (average of 29 cells)	Ratio of long to short arm
1	long arm	33.88		32.16		32.87	
	short arm*	30.03	1.13:1	29.80	1.08:1	29.90	1.10:1
2	long arm	33.30		33.38		33.35	
	short arm	21.24	1.59:1	22.24	1.50:1	21.82	1.53:1
3	long arm	31.44		30.21		30.72	
	short arm	19.12	1.64:1	19.02	1.59:1	19.06	1.61:1
4	long arm	25.99		27.72		27.03	
	short arm	17.13	1.52:1	17.57	1.58:1	17.39	1.55:1
5	long arm	22.46		24.32		23.55	
	short arm	15.40	1.46:1	16.17	1.51:1	15.85	1.48:1
6	long arm	22.91		21.84		22.28	
	short arm	12.96	1.77:1	15.54	1.41:1	14.47	1.54:1
7	long arm	20.72		21.29		21.05	
	short arm	13.73	1.51:1	14.13	1.51:1	13.96	1.51:1
8	long arm	17.13		18.43		17.90	
	short arm	15.09	1.14:1	14.45	1.28:1	14.71	1.22:1
9	long arm	17.26		17.03		17.12	
	short arm	13.41	1.29:1	13.00	1.31:1	13.17	1.30:1
10	long arm	16.49		15.58		15.96	
	short arm	10.97	1.50:1	10.96	1.42:1	10.96	1.46:1
Total length		410.66 microns		414.84 microns		413.12 microns	

* Nucleolar bearing arm

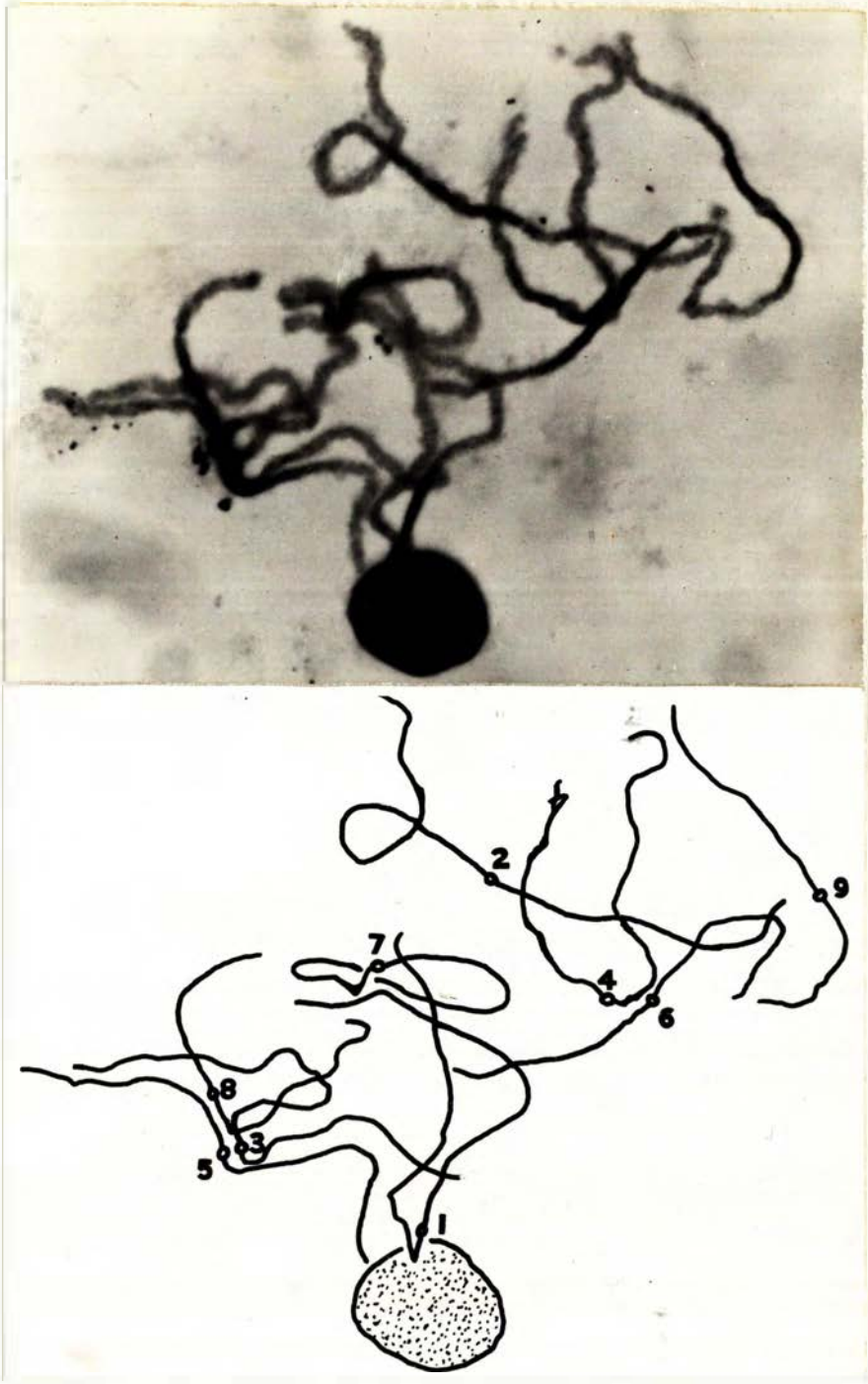


Figure 26. Pachytene chromosomes from line 80 showing stickiness between chromosomes 3 and 5. Only 9 pair are visible in the cell.

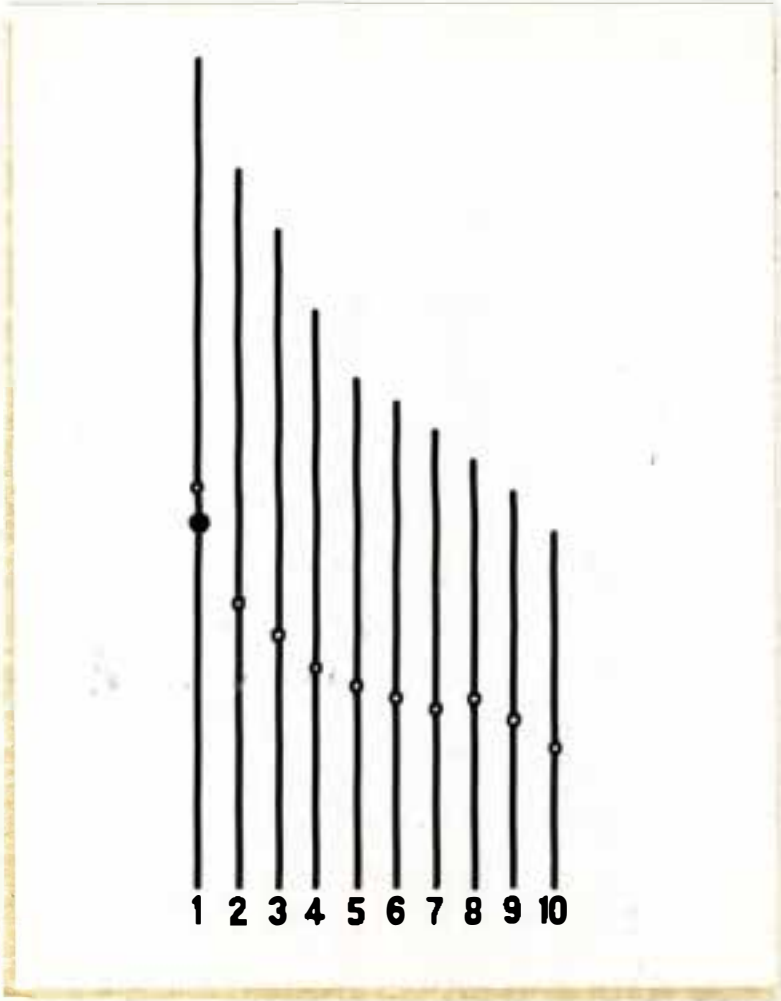


Figure 27. An ideogram of sorghum chromosomes constructed from the mean measurements given in Table 1. The nucleolus attachment point is shown as a solid dot on chromosome one.

Figure 28. F_1 seedlings grown from crosses between Experimental 3 lines. The seedling on the left resulted from a cross between an untreated and a treated line. The dominant red color of untreated Experimental 3 is visible. The seedling on the right resulted from a cross between two treated variants which expressed no red coloration.

seedlings of untreated lines did not appear in the F_1 seedling. Crosses between untreated and treated lines exhibited the red coleoptile of the untreated full sib (Figure 28). The crosses between untreated and treated lines yielded plants that were phenomenally more vigorous and in most cases grew to be 72 inches tall compared to 30 inches in the untreated Experimental 3. No loss of fertility was observed in the F_1 plants grown.

DISCUSSION

The objective of this study was to discover the avenues through which the variation of plant type had occurred. The study of the paired chromosomes was undertaken to determine if homologous pairs were identical in length and chromatin arrangement. Since several of the variants in Experimental 3 resembled one of the ancestral types it was thought possible that chromosomes or parts of chromosomes may have been reduplicated within the chromosome complement, perhaps concentrating the factors for the Sudan grass type. It is doubtful that this could have occurred since chromatin rearrangements would have resulted in multiple association among chromosomes and abnormal pairing when crosses were made between variants and their untreated full sibs.

Had deletions, translocations or inversions taken place within the chromatin of the treated lines these abnormalities could be detected by aberrations at meiosis in F_1 hybrids resulting from crosses between treated and untreated lines. The unpaired areas found in this material were not considered to be indicative of lack of homology since areas of this type were found in treated and untreated lines. In Fritillaria Darlington (3) reported pairing to be variable in all species and incom-

plete in most. He proposed that pairing was based on attractions between single threads; therefore, if the chromatids divided before pairing was completed, further pairing would not take place.

As would be expected from the cytological observations made, the fertility of the F_1 hybrid had not been affected since almost perfect selfed seed-set was obtained after selfing in the greenhouse.

Cytoplasmic inheritance probably was not responsible for propagation of the variations observed in treated material. It was found that characteristics of the variant plants occurred in the F_1 progeny when an untreated plant was used as the female parent. Had this not been true the assumption may have been made that cytoplasm in the egg influenced phenotypic expression of the variant characteristics. The possibility that the variation resulted from gene mutations or cryptic structural changes in the chromatin of treated plants cannot be ignored. Since it was found that colchicine variants exhibited many changed characters it is reasonable to assume that changes must have occurred at many points within the chromatin.

An example of a change in the chromatin was observed in the F_1 plants of Experimental 3. The red coleoptile color characteristic of the untreated material appeared when crosses were made between untreated and treated lines, but green coleoptiles were found in the crosses between true breeding treated lines. It appears that this character was governed by a recessive gene. The phenomenally vigorous growth of the F_1 plants probably cannot be attributed entirely to hybrid vigor. If segregation for height occurs in later generations it would be possible to assume that new genes for this character have occurred in the treated plants.

The ideogram in Figuro 27 does not show complete agreement with the ideogram reported by Longley (10). The major difference between these ideograms is found in chromosome 1. He reported the nucleolus on the long arm of this chromosome; whereas, in material used for this study the nucleolus was found on the short arm.

The secondary associations observed did not appear to influence the pairing relationships or anaphase movements of the chromosomes involved. It was proposed by Kidd, H. J. (from conversation, June 1953) that these associations occurred when the ends of the pachytene chromosomes became entangled during pairing. This position is not supported by the observations made in this study since the material forming the connection between chromosomes did not resemble chromatin in its staining reaction. It was not possible to identify the chromosomes during the later stages of meiosis; therefore, it is not known if secondary associations were peculiar to certain chromosomes pairs. Associations of this type were found in treated, untreated and F_1 plants studied.

SUMMARY

It was proposed that the gross and varied changes in plant type induced by colchicine treatment of a true breeding variety of sorghum was caused by chromosomal deletion or rearrangement. To test this hypothesis a study was made of the meiotic behavior of the chromosomes from lines originating from treated and untreated full sibs and F_1 hybrids of crosses involving variant lines. In this material no irregularities of any kind nor evidence of chromosomal rearrangements or gross deletions were found. Observation of the F_1 hybrid seedlings from Experimental 3 indicated the green coleoptile color of treated lines to

be recessive to the red coleoptiles in untreated lines. Plants in the F_1 generation were extremely vigorous. Because no aberrations were found and recessive characters appeared it is proposed that the colchicine induced variants have resulted from gene mutation or cryptic structural changes in the chromatin.

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